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Urinary Glycol Ether Metabolites in Women and Time to Pregnancy: The PELAGIE Cohort

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Short running head: Women's exposure to glycol ethers and subfertility

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Competing financial interest declaration

The authors declare they have no competing financial interests.

Abstract

Background: Glycol ethers are present in a wide range of occupational and domestic products. Animal studies have suggested that some of them may affect ovarian function.

Objective: To study the relation between women's exposure to glycol ethers and time to pregnancy.

Methods: Urine from randomly selected women in the PELAGIE mother-child cohort who had samples collected before 19 weeks of gestation was tested to measure eight glycol ether metabolites by chromatography coupled to mass spectrometry. Time to pregnancy was collected at the beginning of the pregnancy by asking women how many months they took to conceive. Associations between metabolite levels and time to pregnancy were estimated in 519 women with complete data using discrete-time Cox proportional hazards models to adjust for potential confounders.

Results: Glycol ether metabolites were detected in 6% (for ethoxyacetic acid) to 93% (for phenoxyacetic and butoxyacetic acids) of urine samples. Phenoxyacetic acid was the only metabolite with a statistically significant association with longer time to pregnancy (fecundability OR 0.82; 95% CI: 0.63, 1.06 for the second and third quartile combined; fecundability OR 0.70; 95% CI: 0.52, 0.95 for a fourth-quartile (≥1.38 mg/L) vs. first-quartile concentration (<0.14 mg/L)). This association remained stable after multiple sensitivity analyses.

Conclusion: Phenoxyacetic acid, which was present in most of the urine samples tested in our study, was associated with increased time to pregnancy. This metabolite and its main parent compound, 2-phenoxyethanol, are plausible causes of decreased fecundability, but may also be surrogates for potential co-exposures frequently present in cosmetics.

Introduction

Organic solvents are present in a wide variety of occupational and domestic products including cleaning agents, cosmetics (i.e., makeup, creams, soaps, and deodorants), paints, and varnishes. Several previous studies have reported associations between women's occupational exposure to solvents and fertility impairment, although the results were not all concordant (Mendola et al. 2008). Limitations of such studies include the lack of objective assessment of exposure to specific solvents and failure to study nonoccupational exposure. Biological monitoring of chemicals is a useful alternative as it provides an integrative and objective exposure measurement (Angerer et al. 2007; Calafat et al. 2006).

Among the solvents suspected of impairing female fertility, we decided to focus on glycol ethers (GEs), a family of oxygenated solvents that includes more than 30 different ethers of ethylene glycol and propylene glycol. GEs represented 5% of total volume of solvents in use in France in 2004 (Triollet 2005). Chemical analytical methods were available to measure 8 metabolites of the GEs most frequently used in France at the time of the study (AFSSET 2008) (Table 1). Animal studies have reported that several GEs may affect ovarian function: 2-methoxyethanol (EGME), 2-ethoxyethanol (EGEE), 2-butoxyethanol (EGBE), 2-phenoxyethanol (EGPhE), and 1, 2-bis (methoxyethoxy)ethane (TEGDME) (Multigner et al. 2005). Occupational epidemiologic studies in women have been conducted in the semi-conductor industry, where EGME and EGEE were the main GEs used. In this workplace, GE exposure has been associated with prolonged time to pregnancy (TTP) (Chen et al. 2002; Correa et al. 1996; Eskenazi et al. 1995; Gray et al. 1996) and with prolonged menstrual cycles (Hsieh et al. 2005). However, use of EGME and EGEE has been restricted in France since 1999 (AFSSET 2008). Neither EGBE

nor EGPhE, both frequently present in consumer products and suspected of impairing women's fertility, has ever been studied in women.

The objective of our work was to study the relation between both nonoccupational and occupational exposure to GEs in women and time to pregnancy (TTP).

Materials and methods

Study population

The PELAGIE cohort study, described elsewhere (Garlantezec et al. 2009), included 3421 pregnant women in three districts of Brittany (northwestern France) between 2002 and 2006. Women were recruited by their gynecologists, obstetricians, or ultrasonographers at visits for prenatal care before 19 weeks of gestation and followed through the end of pregnancy. Participants provided informed consent for participation, and the INSERM (National Institute of Health and Medical Research) ethics committee approved the study procedures.

At inclusion, women completed a self-administered questionnaire that covered their family social and demographic characteristics, diet, and lifestyle. They returned the questionnaire by mail to our laboratory together with a urine sample (first morning void) contained in 10-mL test tubes (95x16-mm polypropylene, with wing plug). At reception, urine samples were frozen at -20°C until analysis.

For cost reasons, the biomonitoring study included only a randomly selected sample (n=609) of the women in the PELAGIE Study.

Assessment of time to pregnancy

To assess TTP, we used the answer (in months) to the question "How long did it take you to become pregnant?" from the self-administered questionnaire collected at inclusion. Additional questions asked about contraceptive use, voluntary interruption of contraceptive use (to assess if the pregnancy was planned), fertility medication (to censor at time of conception), and reproductive history (to validate the self-reported TTP by verifying the absence of any interim miscarriage during the TTP). Among women with urine analyses, TTP was available for 519 (85%) (Figure 1). It was unavailable (15%) when the pregnancy was unplanned (n=39) or if it was not clear if the pregnancy was planned or not (n=45), or if the TTP data were missing (n=6).

Chemical analysis of glycol ether (GE) metabolites

Chemical analyses were performed at the Toxicology and Genopathy Laboratory in Lille Hospital (France). GE metabolites were measured by gas chromatography coupled to mass spectrometry. Detection was performed in negative ionization mode with methane in full-scan acquisition between 85 and 152 m/z. This method provided a procedure for the simultaneous analysis of eight alkoxycarboxylic acids, the main metabolites of the GEs in use in Europe at the time of urine collection (Table 1): methoxyacetic acid (MAA), methoxyethoxyacetic acid (MEAA), ethoxyacetic acid (EAA, ethoxyethoxyacetic acid (EEAA), 2-butoxyacetic acid (BAA), n-propoxyacetic acid (PAA), phenoxyacetic acid (PhAA), and 2-methoxypropionic acid (2-MPA). This method, published elsewhere, yields coefficients of variation below 10% at 0.10 mg/L (Labat et al. 2008). The method is linear (r^2=0.99) from 0.05 to 2.0 mg/L for all the alkoxycarboxylic acids with a limit of detection (LOD) equal to 0.05 mg/L.

The influence on urinary measurements of sampling conditions, including transportation time at room temperature, duration of storage at -20°C, and type of acidic stabilizer [hydrochloric acid (HCl) or nitric acid (HNO_3)], and of individual parameters (urinary creatinine level, gestational age at inclusion) was studied in a previous report (Cordier et al. 2012). Experimentation showed that the BAA detection rate decreased with the number of days at ambient temperature when HCl was used as a stabilizer but not when HNO_3 was. Therefore results for BAA are presented only for the subset of 412 women whose samples were stabilized with HNO_3. Statistical analysis showed that creatinine level was associated with MAA, MEAA, EEAA, BAA, and PhAA. Transportation time appeared to influence the levels of BAA, and the detection of 2-MPA. Duration of storage [median: 1090 days (first quartile (Q) 858; third Q 1377)] was associated with levels of MEAA, EEAA, PAA, and 2-MPA. These covariates (creatinine level, gestational age at inclusion, transport duration, and duration of storage between collection and analysis) were therefore considered as potential confounders.

Statistical analysis

Each metabolite with a detection rate less than 50% was categorized in two classes (<LOD and >=LOD). Other metabolites were categorized in three classes (<25th percentile, 25th-74th percentile, >= 75th percentile) and were also modeled as ordinal variables coded using integer values (0, 1, 2) to perform trend tests. Each metabolite with a detection rate greater than 30% was also treated as continuous with a distribution-based multiple imputation (MI) method for handling values < LOD (Jin et al. 2011; Lubin et al. 2004). Assuming a lognormal distribution of each metabolite, this method uses maximum likelihood estimates (MLE) to estimate distribution parameters (Jin et al. 2011; Lubin et al. 2004). Cox proportional hazards models adapted for discrete-time data were used to estimate fecundability odds ratios (fOR) associated with urinary

biomarkers of exposure to GEs metabolites. The fOR estimates the odds of achieving pregnancy in any given month, conditional on not having become pregnant in a previous cycle. When fOR<1, it indicates longer TTP and decreased fecundability. When TTP>12 months (13% of women, n=66), the value was censored at 12 months. When couples sought medical assistance due to difficulty in becoming pregnant and reported TTP<12 months (3% of women, n=16), we censored at their self-reported TTP considering it as a minimal one if they did not have used medical assistance.

Covariates systematically included in the models were well-established infertility risk factors and were self-reported in our study: maternal age (years, continuous), body mass index (BMI) (<18.5 kg/m², 18.5-25 kg/m², ≥25 kg/m²), maternal smoking status when first attempting pregnancy (yes, no), and oral contraceptive use before attempting pregnancy (yes, no).We observed that the probability in our cohort of conceiving in the first 2-3 months was higher for women who had not used oral contraception immediately before attempting pregnancy than for women who did. After 3 months, these two groups no longer differed substantially. This finding, which has also been reported by others (Axmon et al. 2006), led us to stratify our analyses for oral contraceptive use with a stratified Cox model.

We also evaluated other potential covariates: year of inclusion (2002, 2003, 2004, 2005-2006), district of residence (Ille-et-Vilaine, Côtes d'Armor, others), rural/urban residence area, season as a time-varying covariate, self-reported maternal education level (primary/secondary, baccalaureate, post-secondary), coffee consumption at inclusion (≤ once a day, at least twice a day), fish (less than monthly, 1-4 times a month, at least twice a week), and shellfish consumption (less than twice a month, 2-8 times a month, at least twice a week), paternal occupational exposure to solvents at inclusion according to a job-exposure matrix (Ferrario et al.

1988) (unemployed, no, medium, high), and variables describing urine sampling conditions. These variables were to be included in the models if they modified the fOR by 10% or more, but none met this criterion. Additionally, when we observed correlations between metabolites and between TTP and at least one of these metabolites, we ran models that simultaneously included these metabolites.

Correlations between the urinary metabolites were studied with chi-square or Fisher's exact tests, Tobit regression or Spearman rank correlations, depending on the level of detection.

Statistical significance was defined as a p value <0.05.

Sensitivity analyses

The first set of sensitivity analyses investigated the consistency of our findings in light of potential validity issues for TTP studies. As recommended (Joffe et al. 2005; Weinberg et al. 1994), in addition to the main analyses we report estimates from analyses restricted to the 237 primiparous women, excluding 113 women who reported conceiving in the first month, expanding the population to include 39 pregnancies resulting from contraceptive failure (TTP was assigned a value of 0), excluding 37 women seeking fertility treatment, and changing the censoring time from 12 months to 7, 10, or 15 months.

Results

The 519 eligible women had a mean age of 29.4 years when they began attempting to conceive, and their educational attainment was high: around 63% of participants had gone beyond passing their baccalaureate examination (Table 2). Mean gestational age at inclusion was 9.6 weeks (first Q 7.7; third Q 13.3). More than half already had a child, and around 16% had previously

miscarried. Most had used oral contraceptives before trying to conceive and around 7% had undergone fertility treatment. Regular occupational exposure to solvents was reported by 27% of our population (mainly cleaners and helpers, nurses, nurses' aides, hairdressers, and chemists/biologists). The job-exposure matrix classified 16% of women in the medium-exposure category (mainly nurses, nurses' aides, hairdressers, and some cleaners) and 3% in the high-exposure category (mainly chemists/biologists and some production workers). Exposure to solvents during hobbies was reported by 13% of women.

As expected, the entire cohort and the random subcohort were similar for these characteristics (see Supplemental Material, Table S1). The median TTP was 3 months (1^{st}-3^{nd} Q=2-7) (Table 2). Increased parity and unemployment were statistically associated with a reduced TTP (fOR >1.0), and a history of miscarriage was significantly associated with a longer TTP (fOR <1.0).

As Table 3 shows, BAA and PhAA were detected in most of the women's urinary samples and MEAA in more than half. The highest median concentration (estimated from all detected values) was observed for PhAA: 0.48 mg/L.

When we compared the likelihood of detection (i.e., concentration ≥LOD versus <LOD) for metabolites that were detected in less than in <30% of samples (specifically, MAA, EAA, EEAA, MPA, and PrAA) only MPA detection and PrAA detection were significantly correlated, though non-significant associations were also observed for MAA and MPA (p = 0.08) and for EAA and EEAA (p = 0.09) (Supplemental Material, Table S2). When we evaluated correlations between detection of the metabolites above and the concentration of GE metabolites detected in the majority of samples (MEAA, BAA, and PhAA) we found significant correlations between MAA ≥LOD and MEAA concentration, EEAA ≥LOD and PhAA concentration, MPA≥LOD and

the concentrations of MEAA and BAA, and PrAA ≥ LOD and BAA concentration (Supplemental Material, Table S3). However, there were no correlations between MEAA, BAA, and PhAA concentrations (Supplemental Material, Table S4).

Table 4 shows the associations between TTP and each GE urinary metabolite. Fecundability increased with PAA detection (fOR 1.30; 95% CI, 0.94-1.80). PhAA was the only metabolite significantly associated with TTP: a fourth-quartile concentration of PhAA (≥1.38 mg/L) was associated with a 30% decrease (fOR 0.70; 95% CI, 0.52-0.95) in the odds of becoming pregnant each month, compared with the first-quartile concentration (<0.14 mg/L). Moreover, we observed a statistically significant dose-response trend in which fecundability decreased as PhAA levels increased (p-trend=0.02). This result was confirmed by the analysis with PhAA levels treated as a continuous variable (fOR for a 1-mg/L increase in PhAA = 0.95; 95% CI: 0.90, 1.00). Because of the positive correlation between PhAA levels and EEAA detection, we further adjusted for EEAA, but the association with PhAA remained unchanged (fOR for a 1-mg/L increase in PhAA = 0.95; 95% CI: 0.90, 1.00).

The sensitivity analyses of subsets of women and the influence of different censoring times for TTP (Figure 2) yielded similar conclusions for most of the metabolites. The association of longer TTP with a higher PhAA level remained stable. EEAA detection was significantly associated with a longer TTP among primiparous women.

Discussion

The results of this study indicate that concentrations of PhAA, the primary metabolite of EGPhE, in urine samples collected at 9.6 weeks of gestation (on average) were associated with longer

TTP in our study population. This association remained stable after sensitivity analyses. The other GE urinary metabolites measured in our study were not associated with reduced fecundability.

This association may be a chance finding due to multiple comparisons, or could be due to uncontrolled confounding or other sources of bias. However, it has some biological plausibility. The high detection rate of PhAA (93%) in our population is consistent with the frequent use of EGPhE, its main precursor, in cosmetics. EGPhE is also present in pharmaceutical products (AFSSET 2008; INSERM 2006) and biocides (AFSSET 2008), and PhAA is used as a food flavoring agent (FAO-WHO 2002). However, these three sources are probably negligible compared to its cosmetic uses (ANSM 2012). According to French surveys (AFSSET 2008; INSERM 2006), EGPhE was present at the time of our study in at least 50% of perfumes, creams, lotions, makeup, and hair products (except dyes), for example, but only 15 specific individual pharmaceutical products of all drugs (authorized for sale in France) and less than 1% of biocides (AFSSET 2008; INSERM 2006). Moreover, a study by the French Drug and Cosmetic Safety agency (ANSM 2012), estimated that EGPhE intake in cosmetics reached 2 mg/kg/day with daily use of non-rinsed cosmetics (i.e., creams, lotions, powders, perfumes, lipsticks, nail polish, and eye and facial makeup) compared with 0.3 mg/kg/day among drug users and 0.004 mg/kg/day from food (AFSSET 2008; FAO-WHO 2002). EGPhE has been reported in some cleaning products in the United States (Scognamiglio et al. 2011), but this does not seem to be the case in France (AFSSET 2008).

An expert review from the French National Institute of Health and Medical Research (INSERM) stated that EGPhE was one of five GEs likely to impair ovarian function, together with EGME, EGEE, EGBE, and TEGDME (INSERM 1999, 2006; Multigner et al. 2005). The evaluation of

EGPhE was based on a single study in mice, which reported a decreased number of live pups in the exposed group (Heindel et al. 1990). Our results are consistent with the INSERM evaluation for EGPhE, but do not suggest that the other GEs tested at the levels observed in our study have an impact on female fecundability. We have no explanation for the nonsignificant decrease in TTP in association with PAA ≥LOD in urine samples collected during pregnancy, other than chance or uncontrolled bias. To our knowledge, this GE has never been studied in relation to fertility in animal studies.

The only epidemiological studies that have previously evaluated the association between GE exposure and female fertility were performed in the semi-conductor industry (Chen et al. 2002; Correa et al. 1996; Eskenazi et al. 1995; Gray et al. 1996), where the principal GEs used were EGME, EGEE, and 1,2-dimethoxyethane (EGDME). In this occupational setting, prolonged TTP was associated with exposure to these GEs, which was estimated using indirect methods (job title, tasks and expert assessment). However estimates from these studies may be biased due to exposure misclassification or frequent co-exposure to other potential reprotoxic chemicals in this occupational setting (i.e., arsenic, isopropyl alcohol, hydrofluoric acid, phosphorous compounds, and xylene) (Cordier and Multigner 2005). In our study, MAA and EAA (the main metabolites of the three GEs commonly used in the semi-conductor industry) were not frequently detected and were not associated with TTP. However, the regulation of these GEs in Europe since 1999, and the absence from our study of any women working in the semi-conductor industry, where exposure to these GEs is high (mean MAA 39.2 mg/L and EAA 14.2 mg/L at the end of the workday in France) (AFSSET 2008) may explain the low presence of their metabolites in our study population and the lack of associations with TTP.

Measurement of urinary alkoxycarboxylic acids is the method of choice for monitoring occupational exposure to GEs (Laitinen and Pulkkinen 2005; Lauwerys et al. 2007), and when used to monitor the general population provides a measure of exposure from all sources. The metabolites measured in our study have half-lives ranging from 6 to 80 hours, and therefore represent recent exposures only. However, for products used daily in the workplace or at home (such as cosmetics), we believe they may reasonably be assumed to represent regular individual exposure before pregnancy, despite the fact that urine collection took place in early pregnancy. Changes in work environment at the beginning of pregnancy are not likely, since they are not mandatory for occupations such as health care workers, beauticians or hairdressers that constitute the main occupations likely to explain most of occupational GE exposure in our cohort (Cordier et al. 2012). However we cannot exclude possible changes in behavior during pregnancy in particular changes in the use of cosmetics (i.e lotions used to reduce the occurrence of stretch marks although these problems are likely to occur later in pregnancy). Despite the widespread use and potential toxicity of GEs, no one has, to our knowledge, studied their toxicokinetic variability, including during pregnancy. Accordingly, we cannot rule out the possibility that our biomonitoring, which was based only a single urine sample, did not capture total intraindividual variability. Urine PhAA concentrations were significantly associated with only one occupational group, hairdressers and beauticians, in our study population (Cordier et al. 2012). Although we did not collect data on personal cosmetic use, we believe it is likely to explain most of the exposure to EGPhE because only 12 participants reported working as hairdressers and cosmeticians, and the association between PhAA and TTP persisted after excluding them from the analysis (fOR 0.72; 95% CI, 0.53-0.98 fourth-quartile concentration of PhAA compared with the first-quartile).

Although we believe that an effect of EGPhE or its metabolite PhAA on TTP is biologically plausible, we cannot rule out the possibility that urine PhAA concentrations were acting as a proxy marker of exposure to other chemicals, including preservatives frequently present in cosmetics, such as phthalates or parabens. Among phthalates used to be present in cosmetics, di-n-butyl phthalate (DBP) has been associated with a decrease in fertility in female rat (Gray et al. 2006) but this phthalate was banned in cosmetics in France in 2004. A French survey (ANSM 2012) showed that EGPhE and parabens are frequently present together in cosmetics. Parabens were not measured in the urinary samples in our study. Nevertheless, although some parabens (methyl, butyl, benzyl, and isobutylparaben) have been associated with uterotrophic effects in mice or rats at high doses, there is no current evidence that parabens might impair female fertility (Boberg et al. 2010).

Statistical power to detect an effect on fecundability was reduced by our study design: because our population was limited to pregnant women, it underrepresented less fertile couples and possibly led to the underestimation of effects. The short recall time (during the first 4 months of pregnancy) compensated in part for the retrospective collection of TTP data (Zielhuis et al. 1992). The consistent results of the multiple sensitivity analyses we performed, in accordance with recommendations for addressing other possible biases related to this study design (Joffe et al. 2005; Weinberg et al. 1994), reduce, but do not eliminate, concerns about the potential influences of these biases on our results.

Because parity and miscarriage might reflect a certain degree of fertility, we chose in advance not to adjust for them. They might rather be seen as exposure consequences if an association existed and then be considered as intermediate factors in the study of the relation between exposure and subfertility.

In conclusion, among all GEs tested we found evidence of longer time to pregnancy in association with PhAA measured in urine samples collected during pregnancy. PhAA, which was widely detected in our study population, or its main precursor EGPhE are biologically plausible causes of decreased fecundability, but this biomarker may also be a surrogate for co-exposures frequently present in cosmetics. Finally, given the inherent limitations of retrospective TTP studies, further prospective studies on this topic are warranted.

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Table 1: Description of the main products containing glycol ethers in France (2000-2006) and of their main urinary metabolites (source: AFSSET 2008)

Products	Glycol ethers[a]	Metabolites
Cosmetics	EGPhE	PhAA
	EGBE	BAA
	DEGBE	BEAA, BAA
	DEGEE	EEAA, EAA
Drugs	DEGEE	EEAA, EAA
	EGPhE	PhAA
Cleaning agents	DEGEE	EEAA, EAA
	DEGBE	BEAA, BAA
	EGBE	BAA
	PGME	2-MPA[b]
	DEGME	MEAA, MAA
	TEGME	MEAA, MAA
	TEGDME	MAA
Biocides	PGME	2-MPA[b]
	EGBE	BAA
	DEGBE	BEAA, BAA
	DEGEE	EEAA, EAA
	EGPhE	PhAA
Paints, varnishes, inks	PGME	2-MPA[b]
	EGBE	BAA
	DEGBE	BEAA, BAA
	DEGEE	EEAA, EAA
	TEGEE	EEAA, EAA
	EGnPE	PAA

a Glycols ether are presented for each products from the most to the least frequently used b metabolite derived from minor β isomer of PGME

BAA: 2-Butoxyacetic acid; DEGBE: 2-(2-Butoxyethoxy)ethanol; DEGEE: 2-(2-Ethoxyethoxy)ethanol; DEGME: 2-(2-methoxyethoxy)ethanol; EAA: Ethoxyacetic acid; EEAA: Ethoxy-ethoxyacetic acid; EGEE: 2-Ethoxyethanol; EGBE: 2-Butoxyethanol; EGnPE: 2-Propoxyethanol; EGME: 2-Methoxyethanol; EGPhE: 2-Phenoxyethanol; MAA: Methoxyacetic acid; MEAA: Methoxy-ethoxyacetic acid; 2-MPA: 2-Methoxyproprionic acid; PGME: Methoxypropanol; PhAA: Phenoxyacetic acid; PAA: n-Propoxyacetic acid; TEGEE: 2-(2-(2-ethoxyethoxy)-ethoxy) ethanol; TEGME: 2-(2-(2-methoxyethoxy)-ethoxy) ethanol; TEGDME: 1,2-bis (methoxyethoxy)-ethane

Table 2. Median time to pregnancy and crude fecundability odds ratio (fOR) according to various population characteristics

	N (%)	Median (Q1 – Q3) TTP [a]	Crude fOR (95% CI) [a]	p-value
Total	519 (100.0)	3 (2 – 7)		
Year of inclusion				0.48
2002	62 (12.0)	3 (1 – 6)	Ref.	
2003	205 (39.5)	3 (2 – 7)	0.77 (0.55, 1.09)	
2004	188 (36.2)	3 (1 – 6)	0.88 (0.62, 1.25)	
2005-2006	64 (12.3)	3 (2 – 6)	0.88 (0.57, 1.33)	
District of residence				0.84
Ille-et-Vilaine	348 (67.1)	3 (2 – 6.5)	Ref.	
Côtes d'Armor	130 (25.0)	3 (2 – 6)	1.00 (0.78, 1.27)	
Others	41 (7.9)	4 (2 – 7)	0.89 (0.60, 1.32)	
Educational level				0.06
Primary/Secondary	89 (17.2)	5 (2 – 6.5)	Ref.	
Baccalaureate	103 (19.9)	3 (2 – 10)	0.95 (0.67, 1.34)	
Post-secondary	326 (62.9)	3 (2 – 6)	1.26 (0.94, 1.68)	
Missing	1	9	-	
Maternal age (year) at pregnancy attempt				0.21
< 25	61 (11.7)	3 (2 – 8)	Ref.	
25 – 30	206 (39.7)	3 (2 – 7)	1.07 (0.75, 1.52)	
30 – 35	195 (37.6)	3 (2 – 6)	1.34 (0.94, 1.92)	
≥ 35	57 (11.0)	3 (2 – 7)	1.15 (0.74, 1.79)	
Mean ± std	29.4 ± 4.0		-	
Pre-pregnancy BMI (kg/m²)				0.29
< 18.5	39 (7.6)	3 (1 – 5)	1.14 (0.75, 1.73)	
18.5 – 25	399 (77.3)	3 (2 – 6)	Ref.	
≥ 25	78 (15.1)	3 (2 – 8)	0.81 (0.60, 1.09)	
Missing	3	6 (1 – 12)	-	
Mean ± std	22.1 ± 3.4			
Gestational age at inclusion (weeks)				0.25
<10	219 (42.2)	3 (2 – 7)	Ref.	
10 – 13	240 (46.2)	4 (2 – 7)	0.94 (0.75, 1.18)	
> 13	60 (11.6)	3 (2 – 5)	1.27 (0.89, 1.81)	
Mean (+/-sd)	9.6 (+/-2.5)		-	
Parity				0.02
0	237 (45.7)	3.5 (2 – 8)	Ref.	
1	193 (37.2)	3 (2- 6)	1.26 (1.00, 1.59)	
≥ 2	89 (17.2)	3 (1- 6)	1.48 (1.09, 1.99)	
History of miscarriage				0.004
No	434 (83.6)	3 (2 – 6)	Ref.	
Yes	85 (16.4)	4 (2 -10)	0.65 (0.49, 0.87)	

	N (%)	Median (Q1 – Q3) TTP [a]	Crude fOR (95% CI) [a]	p-value
Oral contraceptive use before pregnancy attempt				0.58
No	122 (23.5)	3 (1 – 7)	Ref.	
Yes	396 (76.5)	3 (2 – 6)	1.07 (0.84, 1.38)	
Missing	1	1 (1 – 1)	-	
Maternal smoking at pregnancy attempt				0.22
No	362 (70.8)	3 (2 – 6)	Ref.	
Yes	149 (29.2)	3 (2 – 8)	0.86 (0.69, 1.09)	
Missing	8	6 (4 – 8.5)	-	
Coffee consumption at inclusion				0.94
≤ once a day	366	3 (2 – 7)	Ref.	
At least twice a day	116	3 (2 – 6.5)	0.99 (0.78, 1.27)	
Fish consumption				0.81
Less than monthly	102 (19.7)	3 (2 – 7)	Ref.	
1 – 4 times a month	263 (50.9)	3 (2 – 7)	1.04 (0.79, 1.37)	
At least twice a week	152 (29.4)	3 (2 – 6)	1.10 (0.81, 1.50)	
Missing	2	3 (3 – 3)	-	
Shellfish consumption				0.36
Less than twice a month	353 (68.0)	3 (2 – 6)	Ref.	
2 – 8 times a month	126 (24.3)	3 (2 – 7)	0.98 (0.76, 1.26)	
At least twice a week	40 (7.7)	4 (2 – 8)	0.74 (0.50, 1.12)	
Marital status				0.30
Live alone	5 (1.0)	3 (2 – 6)	0.57 (0.20, 1.64)	
Lives with partner	513 (99.0)	5 (4 – 12)	Ref.	
Missing	1	17	-	
Fertility treatment				
No	482 (92.9)		-	
Yes	37 (7.1)		-	

[a] Computed from women who did not seek medical assistance to achieve pregnancy (n=482)

Table 3. Distribution of urinary levels of GE metabolites, among 519 women

Urinary Metabolites	>=LOD[a] No. (%)	25th percentile (mg/L)	50th percentile (mg/L)	75th percentile (mg/L)	Max (mg/L)	Median (of values>LOD) (mg/L)
Alkoxycarboxylic acids (LOD=0.05 mg/L)						
MAA	146 (28)	< LOD	< LOD	0.06	2.97	0.09
MEAA	289 (56)	< LOD	0.06	0.26	3.90	0.23
EAA	21 (4)	< LOD	< LOD	< LOD	0.62	0.06
EEAA	92 (19)	< LOD	< LOD	< LOD	30.00	0.09
PAA	61 (12)	< LOD	< LOD	< LOD	0.24	0.07
BAA[a]	385 (93)	0.085	0.12	0.16	0.62	0.13
PhAA	484 (93)	0.14	0.38	1.38	36.00	0.48
2-MPA	29 (6)	< LOD	< LOD	< LOD	0.76	0.13

Abbreviations: LOD: limit of detection; MAA: methoxyacetic acid; MEAA: methoxy-ethoxyacetic acid; EAA: ethoxyacetic acid; EEAA: ethoxy-ethoxyacetic acid; PAA: n-propoxyacetic acid; BAA: butoxyacetic acid; PhAA: phenoxyacetic acid; 2-MPA: methoxyproprionic acid.

[a] excluding 107 samples that used chlorhydric acid as stabilizer.

Table 4. Relation between urinary biomarkers of GE metabolites and time-to-pregnancy

Metabolite levels (mg/L)	N	Crude fOR (95% CI)	p-trend	N	Adjusted [a] fOR (95% CI)	p-trend
Methoxyacetic acid (MAA)						
< LOD	373	Ref.		365	Ref.	
≥ LOD	146	1.05 (0.84, 1.33)		142	1.10 (0.87, 1.39)	
Methoxyethoxyacetic acid (MEAA)						0.29
< LOD	230	Ref.	0.31	224	Ref.	
LOD – 0.23	144	1.22 (0.95, 1.56)		140	1.24 (0.96, 1.61)	
≥ 0.23	145	1.12 (0.87, 1.43)		143	1.12 (0.87, 1.45)	
Continuous[b]	519	1.02 (0.78, 1.32)		507	1.03 (0.77, 1.37)	
Ethoxyacetic acid (EAA)						
< LOD	498	Ref.		486	Ref.	
≥ LOD	21	0.90 (0.52, 1.56)		21	0.94 (0.54, 1.63)	
Ethoxyethoxyacetic acid (EEAA)						
< LOD	427	Ref.		416	Ref.	
≥ LOD	92	0.88 (0.67, 1.16)		91	0.88 (0.66, 1.17)	
n-Propoxyacetic acid (PAA)						
< LOD	458	Ref.		447	Ref.	
≥ LOD	61	1.25 (0.91, 1.72)		60	1.30 (0.94, 1.80)	
2-Butoxyacetic acid (BAA)						0.86
1st Q (< 0.09)	103	Ref.	0.96	101	Ref.	
2nd – 3rd Q (0.09 – 0.16)	192	0.98 (0.74, 1.31)		189	0.92 (0.68, 1.24)	
4th Q (≥ 0.16)	117	1.01 (0.73, 1.39)		112	0.96 (0.70, 1.34)	
Continuous[b]	412	1.95 (0.39, 9.79)		402	1.44 (0.28, 7.55)	
Phenoxyacetic acid (PhAA)						0.02
1st Q (< 0.14)	128	Ref.	0.02	126	Ref.	
2nd – 3rd Q (0.14 – 1.38)	261	0.81 (0.63, 1.05)		255	0.82 (0.63, 1.06)	
4th Q (≥ 1.38)	130	0.70 (0.52,0.94)		126	0.70 (0.52, 0.95)	
Continuous[b]	519	0.95 (0.90, 1.00)[c]		507	0.95 (0.90, 1.00)[c]	
2-Methoxypropionic acid (2-MPA)						
< LOD	490	Ref.		478	Ref.	
≥ LOD	29	1.05 (0.66, 1.65)		29	1.10 (0.69, 1.75)	

[a] Adjusted for maternal age at pregnancy attempt (years), pre-pregnancy body mass index (<18.5 kg/m², 18.5-25 kg/m², ≥25 kg/m²), maternal smoking at pregnancy attempt (No, Yes) and oral contraceptive use before pregnancy attempt (No, Yes). [b] Estimated by multiple imputation for non-detects. [c] CI exclude 1.00 before rounding to two decimal places.

Figure Legends

Figure 1. Flow diagram of the study sample.

Figure 2. Adjusted fOR and 95% CIs for association of medium (dotted line) and high (solid line) levels of alkoxycarboxylic acids with time-to-pregnancy (TTP) compared with the lowest levels, according to various sensitivity analyses [a, main population; b, limited to 237 primiparous women; c, excludes 113 women who conceived in the first month of trying; d, includes 39 pregnancies resulting from contraceptive failure (TTP=0); e, excludes 37 women who used fertility treatment; f, censored at 7 months; g, censored at 10 months; h, censored at 15 months].

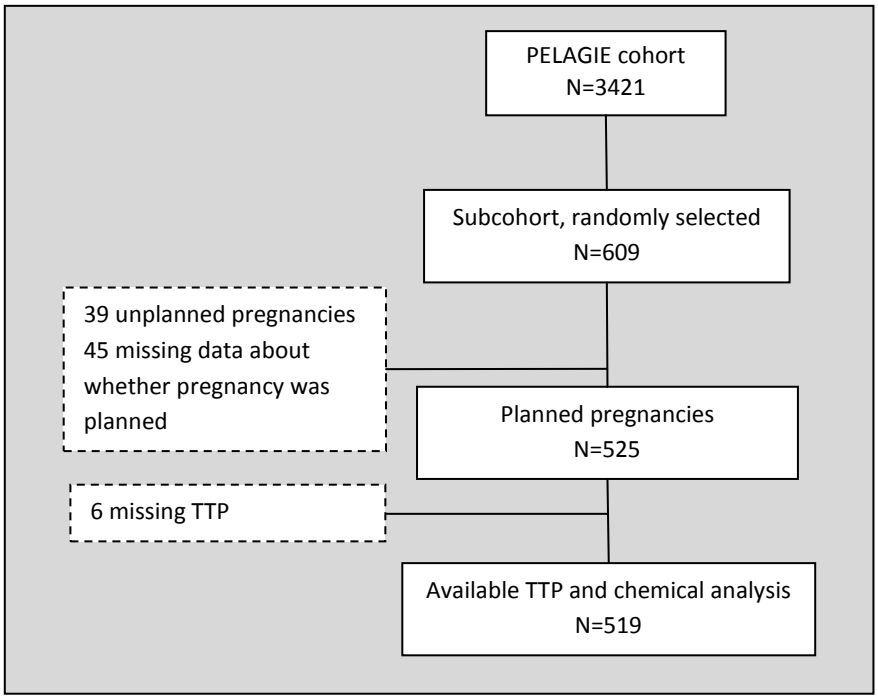

Figure 1. Flow diagram of the study sample.

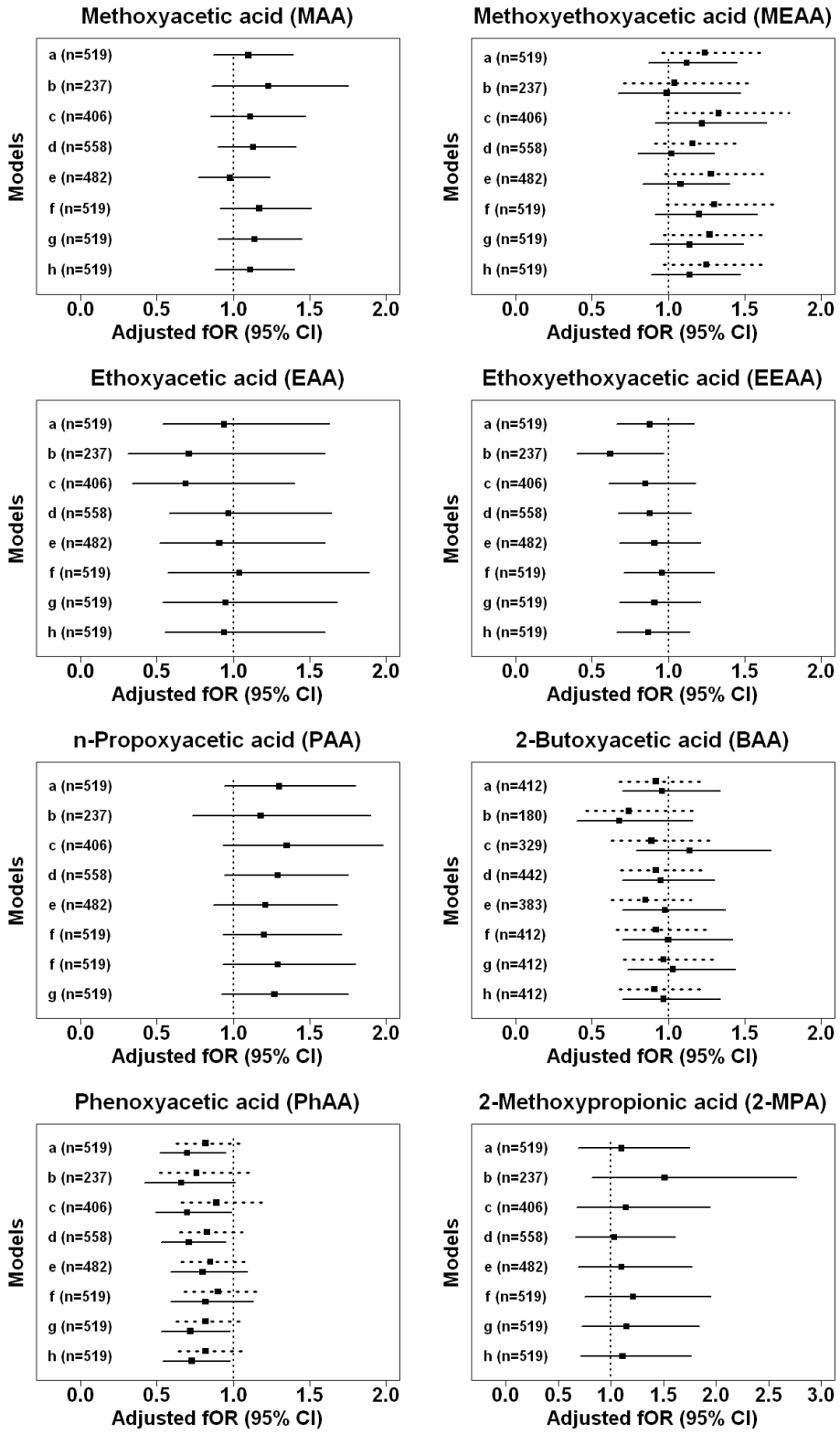

Figure 2. Adjusted fOR and 95% CIs for association of medium (dotted line) and high (solid line) levels of alkoxycarboxylic acids with time-to-pregnancy (TTP) compared with the lowest levels, according to various sensitivity analyses

[a, main population; b, limited to 237 primiparous women; c, excludes 113 women who conceived in the first month of trying; d, includes 39 pregnancies resulting from contraceptive failure (TTP=0); e, excludes 37 women who used fertility treatment; f, censored at 7 months; g, censored at 10 months; h, censored at 15 months].